

22 February 2006

Dr. Seth L. Willey
Acting Regional Recovery Coordinator
US FWS Region 6, Denver, CO

Dear Dr. Willey:

I have completed the review of the King et al. manuscript. Please find the review attached.
Thanks for allowing me to be part of the review process.

Sincerely,

Robert D. Bradley
Associate Professor

Overall, the manuscript is technically and scientifically sound. Actually it represents an ambitious approach to resolving the systematic issue at hand. Philosophically, I disagree with their conclusions, mostly as a result of their interpretations. I will address this and other issues in responding to the series of questions outlined in the review.

- 1). Techniques...I like their approach. The inclusion of 21 microsatellite loci is outstanding. In addition, they examined the mitochondrial cytochrome b gene and approximately one-third of the mitochondrial control region. If any criticisms can be leveled, it would be that they looked at only a "short segment" of the control region. The control region has different domains that evolve at different rates. In other words you might recover different information from the "front", "middle", or "back" regions. This is a minor criticism and is more for thought than anything. Overall, the appropriate markers and methods were used.

I am not sure about the validity of F_{st} values for distinguishing among subspecies. This may be an over extension of the statistic.

- 2). Conclusions...This is the gray area that unfortunately is at the heart of the study. The authors definitely demonstrate that genetic variation exists between populations and that the variation corresponds to taxonomic units. The problem is in how to interpret that variation. All of the analyses show that the various subspecies form discrete units and that there is little or no gene flow between them. However, the most robust analysis, the maximum likelihood analysis, shows very little resolution (extremely short branch lengths). Also there are no support values for any of the clades and it may be that the large clade of interest would collapse.

In my opinion, here is crux of the problem. If you randomly picked populations that were somewhat isolated (genetically or by geographic distance) you might find evidence of little or no gene flow, especially with microsatellite data. It is too difficult to determine if this is the case in this instance. Given that you would fall back to the position – how different are the groups genetically? Based on the branch lengths shown in the maximum likelihood analysis, I would conclude that they are genetically different but the level of differentiation is extremely small. Does this warrant recognition as a separate subspecies? Probably not, in my opinion. I would agree that the authors have demonstrated that certain populations are genetically different but again this could be said for populations within most species boundaries.

- 3). Two populations...The authors have shown a northern and southern genetic division in *preblei*. Again the answer is similar to question number 2 – I think you would expect this in most populations.
- 4). Alternative interpretations...The question is where do you draw the line – at populational differences or at a higher level. I think the authors have demonstrated difference at the populational level but I am not sure that the case can be made at a higher taxonomic level.
- 5). Additional analysis...The choice of analyses is pretty sound. I would recommend a Bayesian analysis with clade probability values. This would provide support for the various clades and would help resolve the concern with their maximum likelihood analysis.
- 6). Conflicts...I am not sure the two conflict as much as the authors state. Both show similar groupings of taxa. The differences lie in where you draw the line and infer either populations or subspecies. In many ways, this is a philosophical debate and may not be resolvable.